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Small leucine-rich proteoglycans of the cartilage and their potential role in tissue repair

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The extracellular matrix (ECM) of the articular cartilage is composed of a variety of molecules synthesized by the chondrocytes. These molecules include fibril-forming collagens, large proteoglycans (PGs), such as aggrecan, and small leucine-rich PGs (SLRPs), such as biglycan and decorin. Chondrocytes also express cytokines and growth factors by which the cells respond to signal from the environment by changing the expression levels of the ECM molecules. It is believed that chondrocytes maintain homeostasis by balancing catabolic and anabolic events and thus, preserving the integrity of the ECM. Molecules that regulate the effects of growth factors and cytokines must be present in the matrix in order to balance their activities. Such regulatory molecules are known to be present in the ECM and they regulate matrix assembly and homeostasis by binding to matrix molecules or inhibiting growth factors, cytokines and enzymes. One of these molecules is believed to be the family of SLRPs. The structurally similar biglycan and decorin play regulatory roles in the assembly of the ECM by binding to different matrix molecules, including fibrillar collagens, fibronectin and the members of the TGF-beta growth factor family. While decorin plays a key role in regulating collagen fibril formation, biglycan plays a crucial role in the assembly of the pericellular matrix network. In osteoarthritis (OA), the delicate balance between catabolic and anabolic processes is broken, and the degradative processes are not well-balanced by repair attempts, resulting in massive loss of matrix molecules. We previously reported that the expression of both biglycan and decorin is increased in degenerated and OA cartilage. We have also shown that the expression of these SLRPs is upregulated by growth factor/cytokine effects (Il-1, IL-6, TGF-beta, and OP-1); their expression is either upregulated by the cytokine or increased after the removal of the cytokine. Thus, we propose that the increased expression of growth factors and the SLRPs is part of the attempt by the chondrocytes to repair the matrix. However, how decorin and biglycan is involved in the repair process is still to be determined. In this study, the potential consequences of the presence of elevated levels of decorin and biglycan, including their role in regulating growth factor effects and their direct effect on chondrocytes is determined.

Human ankle joints from 20 donors with no known joint disease (age 65-75 years old) were received from the Gift of Hope Human Organ and Tissue Donor Network and used with the permission of the Institutional Review Board. All cartilages were normal by gross morphological examination (Collins Grade 0-1). Chondrocytes were released from the cartilages by sequential enzymatic digestion and cultured in confluent monolayers or encapsulated in alginate beads. Cell binding assays: Biglycan and decorin (Sigma) were labeled with Rhodamine-red (RR) according to the manufacturer's instructions (Molecular Probes). Cells were cultured overnight in chamber slides, and then were subjected to RR-labeled biglycan or decorin at different concentrations for 30 min to 3 hr at 37°C. Fluorescent signal was observed with a Nikon Eclipse E600 microscope and images linked to a computer equipped with Metamorf software used for image analyzis. Competition assays were performed using RR-labeled biglycan or RR-labeled decorin in competition with non-labeled biglycan, non-labeled decorin, epidermal growth factor (EGF, Sigma), EGF receptor blocking antibody (Sigma), or EGF receptor antagonist, Tyrphostin (Sigma). Fluorescent signal was observed with the Nikon Eclipse E600 microscope, listed above. For signaling studies, chondrocytes were cultured in monolayer and treated with different concentrations of biglycan or decorin between 10 minutes and 24 hours. Cells were also treated with EGF and inhibitors of several signaling pathways (Calbiochem). Cells were lyzed and signaling molecules as well as cyclin-dependent kinase inhibitors p21 and p27 were detected using specific antibodies (Cell Signaling) on Western blots. For gene expression studies, chondrocytes were cultured and treated as above for 2-24 hours. The cells were lyzed and total RNA was isolated. Expression levels of aggrecan, collagen type II, p21, p27, and certain matrix metalloproteinases (MMPs) were determined by RT/PCR using GAPDH as a control. Proteoglycan synthesis and accumulation in alginate bead cultures (24-96 h) were determined by sulfate incorporation and by DMMB assay, respectively, in the presence or absence of biglycan or decorin. Data were normalized to cell number determined by Picogreen DNA assay (Molecular Probes). Interaction of biglycan and decorin with growth factors was monitored by immunoprecipitation assays. Biglycan and decorin was incubated with TGF-beta or OP-1 (BMP-7) and the complex was precipitated with specific antibodies to these SLRPs. Western blot hybridization was used to detect the co-precipitated growth factors. ELISA assay was used to calculate the molecular ratio between small PGs and growth factors. Regulation of the effect of growth factors was achieved by treating chondrocytes, cultured in alginate beads, with biglycan, decorin, TGF-beta or OP-1 alone and with the combination of the small PGs with the growth factors. Proteoglycan synthesis and accumulation was quantified. Statistical analyzes were performed by ANOVA.

Chondrocytes express biglycan and decorin, and these molecules are deposited in the matrix. Although, both decorin and biglycan can be detected in both the pericellular and extracellular matrices, the majority of decorin is localized in the extracellular matrix on the collagen fibers, while the majority of biglycan resigns in the pericellular matrix. In OA tissue, both SLRPs are expressed and accumulated in elevated amounts around the clusters formed by those cells that play active roles in the repair attempt. These newly synthesized biglycan and decorin molecules thus have the opportunity to influence the repair process as well as the action of growth factors. Our data showed that both biglycan and decorin was able to bind TGF-beta and OP-1 in an approximate 1:2 molar ratio. While both treatment with TGF-beta and OP-1 upregulated PG synthesis and resulted in an accumulation of PGs in alginate beads, the combination of these growth factors with biglycan or decorin abolished the anabolic effect of the growth factors. When biglycan or decorin were applied alone, these SLRPs exerted an inhibitory effect on PG synthesis and accumulation, thus these molecules have a direct effect on chondrocytes. When RR-labeled decorin or biglycan were applied in cell culture, these molecules were able to bind to the cells in a concentration- and time-dependent manner. Interestingly, biglycan completely abolished the binding of the RR-decorin and vice versa, thus both molecules bind to the same receptor. Competition of biglycan and decorin with the epidermal growth factor (EGF), an EGF receptor blocking antibody or Tyrphostin was concentration-dependent, and led to almost complete abolishment of the fluorescent signal. This proves that the majority of biglycan and decorin binds to these cells through the EGF receptor. Both biglycan and decorin was found to be signaling through the MAPK (ERK) and the PI3K (Akt) pathways, similar to EGF, the natural ligand of the EGF-receptor. Signals were inhibited by pathway-specific inhibitors. Biglycan and decorin also activated p21 and p27, both factors known to promote cell cycle arrest. Treatment of the cells with either biglycan or decorin resulted in a significant decrease in the expression levels of aggrecan and type II collagen. This goes parallel to the inhibition of PG synthetic rate and accumulation measured in alginate bead cultures. On the contrary, the message levels of MMP-3 and -13 were upregulated.

In this study, we demonstrate for the first time that biglycan and decorin may play an important role in regulating the effects of growth factors in cartilage. This effect can be exerted through growth factor binding and also through direct binding of these SLRPs to specific cell surface receptors of chondrocytes. Receptor binding can then result in the activation of specific intracellular pathways and regulation of certain gene expression levels. The major receptor for biglycan and decorin is most likely the EGF receptor, and the MAPK (ERK) and PI3K (Akt) pathways are the ones that are activated by both small PGs. According to our findings, when biglycan and decorin are used in combination with growth factors, the anabolic effect of these factors on PG synthesis is abrogated. In turn, the binding of decorin or biglycan to the EGF receptor results in downregulation of the expression levels of the aggrecan and collagen type II genes. This in turn, results in reducing matrix deposition around the cells. This may imply that these SLRPs, which are upregulated by TGF-β and OP-1, may serve as regulators of the effects of growth factors by imposing an opposite effect on the expression levels of the major ECM molecules. The ability of the SLRPs to upregulate MMP-3 and MMP-13 suggests not only an anti-anabolic but also a catabolic role for this molecule. This, again, is an opposite of the effect of growth factors on these enzymes. The upregulation by biglycan and decorin of cell cycle inhibitors p21 and p27 that inhibit cell proliferation may additionally counteract the effect of growth factors. In summary, these results suggest that the presence of increased amounts of biglycan or decorin in OA cartilage may interfere with the repair process by negatively regulating the effect of growth factors on matrix deposition and cell proliferation. This role of these SLRPs may be very important in normal homeostasis to allow cells to work within a regulated environment. In OA cartilage, where the loss of matrix is extensive, this regulatory effect may also be important for a certain extent, but it also may contribute to the inability of growth factors to repair the matrix properly.